1874-Pos
Highly Variable Microtubule Assembly Dynamics Reflect Near-Kilohertz Kinetics: Evidence Against Traditional Linear Growth Theory
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Microtubules are intracellular polymers that dynamically grow and shorten at their ends via the stochastic addition and loss of alpha-beta-tubulin heterodimers. The kinetics of tubulin assembly are central to the regulation of microtubule dynamics by microtubule-associated proteins and therapeutic drugs. Previously, rates of tubulin subunit exchange at the ends of growing microtubules have been estimated using a linear growth theory that assumes tubulin dissociation occurs at a constant rate regardless of the free tubulin concentration. However, by measuring the variance of microtubule assembly at the nanometer scale, we find that stochastic tubulin dynamics are an order of magnitude faster than previously estimated. This discrepancy is explained by molecular-level simulations showing that the tubulin dissociation rate during microtubule growth is not constant, but rather should increase with increasing tubulin concentration. This indirect effect is due to a concentration-dependent bias in simulated microtubule tip structure, as has been experimentally observed. Our analyses indicate that the published tubulin subunit addition and loss rates at growing microtubule ends *in vitro* have been consistently underestimated in the literature: the variance in the assembly rate *in vitro* is too high to be consistent with the previous low kinetic rate estimates, and we conclude that both tubulin addition and tubulin loss events occur on the millisecond time scale, far faster than the previously believed 10-1000 millisecond scale. More generally, we demonstrate that the fixed off rate originally used in the linear growth theory of Oosawa and assumed in most subsequent models, fails to describe the behavior of self-assembled polymers having both lateral and longitudinal bonding interactions between subunits.

1875-Pos
Filament Localization with Nanometer Accuracy
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Recent developments in optical microscopy and nanometer tracking of single fluorescent molecules (or alternative subresolution particles) have greatly enhanced our understanding of biomolecular processes in vivo and in vitro. In particular, fitting the intensity profiles of nanometer-sized objects to 2D-Gaussian models allows their two-dimensional localization with an accuracy in the one-nanometer range, primarily only limited by the number of photons collected. Here, we present a novel algorithm which adapts 2D-Gaussian models to precisely determine the contour, as well as the end points, of curved filaments whose structures are characterized by subresolution diameters and micrometer lengths. Utilizing surface-immobilized microtubules (diameter of 25 nm, densely labeled with fluorophores) we demonstrate positional accuracies of ~2 nm and ~15 nm when localizing the center line and the end points of the filament, respectively. We report on the application of the algorithm to determine (i) the dynamics of microtubule polymerization/depolymerization and (ii) the speed of microtubules gliding over motor-coated surfaces. Combined with methods to measure nanometer heights above substrate surfaces (such as fluorescence interference contrast or parallax), our algorithm - which is also readily applicable to fluorescently labeled actin or DNA/RNA filaments - presents a promising tool for optical 3D-nanometry.

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The Effect of Human Microtubule-Associated-Protein Tau and Ionic Strength on the Assembly Structure of Microtubules: Synchrotron X-Ray Scattering and Binding Assay Study
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Microtubules (MTs), a major component of the eukaryotic cytoskeleton, are 25 nm protein nanotubes with walls comprised of assembled protofilaments built from αβ heterodimeric tubulin. In neural cells, different isoforms of the microtubule-associated-protein (MAP) tau regulate tubulin assembly and MT stability. Using synchrotron small angle x-ray scattering (SAXS) and binding assay, we examine the effects of human MAP tau on the assembly structure of taxol-stabilized MTs. We find that tau regulates the distribution of protofilament numbers in MTs as reflected in the observed increase in the average radius of MTs with increasing the tau/tubulin molar ratio. Further, we describe that tau-MT interactions are mediated to a large extent via electrostatic interactions: the binding affinity of tau to MTs is ionic strength dependent. Supported by DOE DE-FG02-06ER46314, NSF DMR-0803103, NIH NS35010, NIH NS13560. (Ref) M.C. Choi, S.C. Feinstein, and C.R. Safinya et al. *Biophys. J.* 97; 519 (2009).

1877-Pos
A Fluorescent GTP Analogue as a Single Molecule Fluorescence Label of Microtubules
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Microtubules are cytoskeletal polymers which play a role in cell division, cell mechanics, and intracellular transport. Dynamic studies of microtubule function make use of fluorescent labels via antibodies, paclitaxel, and direct attachment to the tubulin protein. However, these labels suffer from drawbacks such as transient labeling, occlusion of functional sites on the microtubule surface, or structural non-specificity. Here we report a new, complementary fluorescent labeling technique that avoids these drawbacks. A fluorescently modified GTP analogue is used to polymerize microtubules from tubulin dimers. This GTP analogue binds selectively to the exchangeable GTP-binding site (E-site) on the tubulin dimer, which is available only during polymerization. The E-site affinity of this GTP analogue is about 100 fold weaker than that of GTP. Because this labeling technique places a bright fluorophore at a defined location within the microtubule lattice, it may facilitate observations of microtubule dynamics with increased precision.

1878-Pos
Kinesin-Calmodulin Fusion Protein as a Molecular Shuttle and Marker for Plus End of Microtubule
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In the present study, we have demonstrated that the novel molecular shuttle with reversible cargo-loading system by using calmodulin (CaM) and M13 peptide. We designed a kinesin (K560) chimera protein with CaM fused at the C-terminal tail region of K560 (K560-CaM). K560-CaM was expressed using an Escherichia coli expression system and purified. We successfully observed that K560-CaM transported quantum dot-conjugated M13 peptide along the microtubule in the presence of Ca2+ by the total internal reflection fluorescence microscopy. Reversible Ca2+dependent cargo-loading system was achieved by changing the Ca2+ concentration in the flow cell. K560-CaM was adsorbed onto the fluorescently unlabeled microtubule adhered on the glass surface in flow cell using non-hydrolyzable ATP analogue, AMP-PNP which stabilize the microtubule binding state of kinesin. Subsequently, Qdot-M13 was added in the presence of Ca2+ to be loaded on K560-CaM adsorbed on the microtubule. The fluorescence of Qdot-M13 loaded onto K560-CaM along a microtubule was observed after washing excess unbound Qdot-M13. When the Ca2+ solution in the flow cell was replaced by the Ca2+ free solution, Qdot-M13 was unloaded. Even after the several times alternate exchange of the solution in the flow cell with Ca2+ and EGTA solutions, the K560-CaM adsorbed onto microtubule by AMP-PNP showed stable Ca2+ dependent cargo loading. When excess ATP was added into the flow cell, K560-CaM-Qdot started to move along the microtubule. Interestingly, 145 seconds later, K560-CaM-Qdot accumulated at plus end of microtubule and showed fluorescent clumps as marker for plus end of microtubule.

1879-Pos
High Resolution Structural Characterization of the Human Spastin Protein and its Complex With Tubulin
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Hereditary spastic paraplegia (HSP) is a motor neuron disease caused by a progressive degeneration of the motor axons of the corticospinal tract. A number of different proteins have been implicated in the pathology of HSP, including mitochondrial proteins, a kinesin motor, spartin and spastin. Of the autosomal dominant cases of HSP, approximately 40% are caused by point mutations or exon deletions in spastin. Because of spastin's significant role in the development of HSP, there have been an increasing number of studies on this protein and its role in axonal degeneration. Recent studies have shown that spastin has a microtubule severing activity, and that this activity is linked to the manifestation of neurological disorders in Drosophila. Until recently very little structural information has been available for the spastin protein, and the mechanism by which it severs microtubules remains elusive. The goal of this study is to